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In vitro determination of the antimicrobial potential of homemade preparations based on medicinal plants used to treat infectious diseases

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ABSTRACT

The majority of the population in developing countries uses plants or plant preparations in their basic health care. Many plant species used nowadays in folk medicine have been proved to have antimicrobial properties. However, several factors, such as incorrect preparation of the plants, can interfere with the effectiveness of the treatment. The purpose of this study was to assess the use of homemade preparations of medicinal plants in the treatment of infectious diseases, by *in vitro* determination of their antimicrobial potential. Based on recipes elicited by questionnaires that were previously applied to a participant population, the samples were prepared in a similar manner and analyzed by the agar diffusion method. Members of 41 families, whose children attend a center of education that serves several needy communities in the city of Fortaleza (Ceará, Brazil), were interviewed; of these, 97.6% said they had used herbal therapy as a means to treat infectious diseases. In replies to a total of 39 questionnaires, 97 different homemade preparations of medicinal plants were cited. Out of 45 samples subjected to an *in vitro* assessment of antimicrobial activity, 25 (55.6%) had some inhibitory effect on the growth of at least one of the microorganisms used. Most of the plants with known antimicrobial properties and cited by respondents showed variations in their *in vitro* activity, according to the manner in which they were prepared.

Keywords: Medicinal plants. Products with Antimicrobial Action. Traditional Medicine. Homemade preparation.

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INTRODUCTION

While modern medicine is well-developed in most parts of the world, the World Health Organization recognizes that much of the population in developing countries depends on traditional medicine for their primary care: 80% of the population use traditional practices in their basic health care and, out of these, 85% use medicinal plants or herbal preparations (OMS, 2000).

The use of medicinal plants in health care is as old as humanity. It is now known that primitive peoples constantly sought medicines from the plant kingdom to alleviate human suffering caused by disease or accidents. For thousands of years, people have been discovering and using plants and chemical substances derived from them to cure diseases. In Brazil, the use of these compounds for pharmacological purposes has grown gradually (Amorozo, 2002; Botsaris, 2007).

Brazil is rich in terms of biodiversity, in sharp contrast to the large pockets of poverty that exist in all regions, where the poor population – besides having difficulties in obtaining conventional medicines – get sick at a much higher rate. The deep socio-economic inequalities, particularly in the Northeastern region, are reflected in the important role that infectious diseases, especially intestinal infections and respiratory infections, still have as a major cause of illness and death, especially among children (Torres et al., 2005).

Common microorganisms such as *Escherichia coli*, *Candida albicans, Staphylococcus aureus*, *Pseudomonas aeruginosa* and some *Salmonella* strains are important etiologic agents for many diseases in the community (Ruhnke, 2006; Pires et al., 2007; Marcus et al., 2008; Nevet et al., 2010; **Reddy, Shaw & Crump, 2010**). In recent years, some strains of these organisms have become a leading cause of infectious disease morbidity and mortality around the world, because of the emergence of strains resistant to many antibiotics; for example, methicillin-resistant *S. aureus* (MRSA) and carbapenem-resistant *P. aeruginosa* (Pichereau & Rose, 2010; Vitkauskienė et al., 2010).

The development of microbial resistance to existing antibiotics is a constant cause for concern in the treatment of infectious diseases. In recent decades, the pharmaceutical industry has produced a large number of new antibiotics, but resistance to these drugs develops rapidly, making it impossible to foresee how microorganisms will respond to new antimicrobial drugs in the future (Rossi & Andreazzi, 2005). Thus, the search for plant products with high antimicrobial activity is of the utmost importance, as it provides an opportunity to find compounds with much greater activity than the drugs available today. Moreover, in light of the fact that bacteria with multiple resistance to antibiotics pose a challenge to the treatment of infections, the need to find new substances with effective antimicrobial properties is evident (Pereira et al., 2004).

Plants of the Brazilian biomes have long been used in traditional medicine by the local population to treat a variety of diseases, including those caused by microorganisms, especially bacterial and fungal infections. The antimicrobial activity of several species of medicinal plant has been researched, both in Brazil (Alencar et al., 2005; Loguercio et al., 2005; Botelho et al., 2007; Botelho et al., 2008; Pereira et al., 2010) and other countries (Konning et al., 2004; Wannissorn et al., 2005; Biradar et al., 2008; Al-Bayati & Al-Mola, 2008; Van Vuuren & Naidoo, 2010).

According to Matos (2002), **empirical herbal medicine**, conducted in Brazil by lay professionals, is usually done in the form of homemade preparations, without following any criteria of safety and efficacy. This rather careless practice in the use of medicinal plants can, due to insufficient knowledge on the subject or the idea that "if it's natural, then even if it does you no good, it won't do you any harm," lead to unwanted results, such as a lack of any response to the medication or even serious cases of poisoning, generally stemming from the frequent use of combinations, whether plant x plant or plant x medicine (Medeiros Filho et al., 1997; Torres et al., 2005).

The aim of this study was to discover the main plant species used for medical purposes by families whose children attend a center of education that serves several needy communities in the city of Fortaleza (Ceará, Brazil), and to test *in vitro* the antimicrobial potential of homemade preparations of medicinal plants used frequently by this population for the treatment of infectious diseases.

MATERIALS AND METHODS

Population under study

The participant population was composed of families whose children were attended at a center of education that serves several needy communities in the city of Fortaleza (Ceará, Brazil). This population has low purchasing power and thus encounters obstacles to purchasing allopathic medicines and has poor access to hospital facilities for laboratory exams and the dispensing of medicines, contributing to the high frequency with which this population turns to herbal medication.

Application of the questionnaire

Firstly, the project and its goals were introduced to families with children attending the children's education center. Those families who agreed to participate signed a statement of consent authorizing the interviewers to apply the questionnaire in their homes. Data collection was conducted through interviews, based on a semi-structured questionnaire composed of 28 questions designed to survey socio-economic and ethnobotanical data. The answers provided the researchers with information about the main medicinal plants used by the local population, the popular names of the plants, the parts of the plant most commonly used, indications, methods of preparation and methods of administration, among other topics. A criterion of the study was that only one member of each family was interviewed (generally the one responsible for the family's care), who provided the information needed to complete the questionnaire during home visits.

Plant Material

The plant samples used in the analyzed preparations were obtained at traditional market places in the city of Fortaleza (Ceará, Brazil), from January to April 2007. The formulations were prepared as reported by the interviewees and then tested against the microbial strains.

Determining the antimicrobial potential

To determine their antimicrobial potential, herbal preparations were selected that — according to the answers to the questionnaires — were used to resolve the following signs and symptoms that can be associated with a possible condition of infection: fever, body aches, sore throat, flu, catarrh (used as an expectorant to expel phlegm), cough, "runny nose" (coryza), shortness of breath, abdominal pains, diarrhea, "inflammation" and "kidney infection."

The *in vitro* antimicrobial potential of the homemade medicinal plant preparations used by the population under study was assessed at the Laboratório de Pesquisa em Microbiologia Aplicada do Departamento de Análises Clínicas e Toxicológicas da Faculdade de Farmácia, Odontologia e Enfermagem da Universidade Federal do Ceará (UFC), Brazil.

Microbial strains

The following five reference microbial strains originating from the Laboratory of Reference Materials of the **Fundação Oswaldo Cruz** (FIOCRUZ) were used: *Staphylococcus aureus* ATCC 6538P, *Escherichia coli* ATCC 10536, *Candida albicans* ATCC 10231, *Pseudomonas aeruginosa* ATCC 9027, and *Salmonella enterica* subsp. *enterica* serovar Choleraesuis ATCC 10708.

Antimicrobial assay

The antimicrobial potential of the homemade preparations of the medicinal plants was determined by

the disk diffusion method, adapted from Valgas et al., (2007). Isolated colonies of the above strains growing on the surface of a solid medium were suspended in BHI broth (Merck, Darmstadt, Germany), which was incubated at 35° C until reaching an opacity visibly equivalent to 0.5 on the McFarland scale (approximately 10^8 CFU/mL).

With sterile swabs, these suspensions were spread on the surface of Mueller-Hinton agar (Merck¸ Darmstadt, Germany) in three directions, so that a confluent and homogeneous growth was obtained. After 5 minutes, wells (6mm in diameter) were made in the agar with a sterile punch and 100 µL of a plant preparation was placed in each well. Commercially-available antibiotics (0.3 mg/mL amikacin for bacteria and 0.5 mg/mL ketoconazole for yeast) were used as positive controls (inhibition of microbial growth) and the sample solvent (sterile distilled water) as the negative control (no inhibition of microbial growth).

After incubation at 35ºC for 18h, all plates were examined for growth inhibition zones (haloes), whose diameters were recorded. Clear haloes around the wells indicated the presence of antimicrobial activity. All tests were performed in triplicate.

Ethical issues

This study was submitted to and approved by the Ethics Committee of Universidade Federal do Ceará, Brazil (res. 242/06).

RESULTS

Socio-economical data

From June to November 2006, representatives of 41 families were interviewed and filled out a semi-structured questionnaire. Most of the interviewees were women (95.1%), mainly in the age range 18-37 years (78%), who had completed elementary school (75.6%). The participating families had low income levels (82% had no more than twice the national "minimum wage", a government-defined poverty line), and almost all of the respondents (97.6%) said they had sought medical attention from Brazil's *Sistema Único de Saúde* (the universal National Health System).

Ethnobotanical data

Concerning the use of medicinal plants, 97.6% (39) affirmed that they use or had already used some kind of homemade plant preparation at some time in their lives. Cultural heritage was cited as being the greatest source of learning about plants with medicinal uses, 61.5% stating they had learned from older generations (mostly parents and grandparents), while healthcare professionals were cited by only 10.2% of the questionnaires.

The preparation of "remedies made from plants" was almost entirely the responsibility of female members of the family: mothers (71.8%) and grandmothers (10.2%). It was observed that *raizeiros* (market-stall herb sellers) played an active role in the supply of medicinal plants to the population under study: 74.4% of the respondents acquired plant materials by purchase. Home-growing and donations of plants by neighbors had a lower share: 28.2% each.

Phytotherapy enjoyed high credibility among the respondents; of those who regularly used medicinal plants, 82.1% always found treatment with herbal medicines satisfactory and 92.3% affirmed that they had never experienced any undesirable effects of using these medicines.

A total of 97 plant preparations were cited by the 39 families that used them, at an average of 2.5±1.6 and a variation from 1 (38.5%) to 7 (2.6%) preparations per questionnaire. It was found that the knowledge of most of the respondents was restricted to a few plants (Table 1). Most species cited by the population were used with more than one therapeutic indication (Table 2).

Table 1. Plant species used by the interviewed families*

POPULAR NAME	SCIENTIFIC NAME	FAMILY	CITATION	
			Nº	%
"Abacaxi"	*Ananas sativum* (L.) Merr.	Bromeliaceae	1	0.7
"Agrião"	*Nasturtium officinale*	Cruciferae	1	0.7
"Alfavaca"	*Ocimum gratissimum* L.;	Labiatae	8	5.6
"Alho"	*Allium sativum* L.	Liliaceae	5	3.5
"Anador"	Justicia pectoralis	Acanthaceae	1	0.7
"Aroeira"	*Myracrodruon urundeuva* All.	Anacardiaceae	4	2.8
"Arruda"	*Ruta graveolens* L.	Rutaceae	1	0.7
"Beterraba"	*Beta vulgaris* L.	Chenopodiaceae	6	4.2
"Boldo"	*Peumus boldus* Mol.	Monimiaceae	9	6.3
"Camomila"	*Matricaria chamomilla* L.	Compositae	1	0.7
"Capim-santo"	*Cymbopogon citratus* Stapf.	Gramineae	8	5.6
"Cebolinha branca"	*Allium ascalonicum* L.	Liliaceae	8	5.6
"Cidreira"	*Melissa officinalis*	Lamiaceae	11	7.7
"Corama"	*Kalanchoe brasiliensis* Camb.	Crassulaceae	5	3.5
"Cumaru"	*Amburana cearensis* (All.) A. C. Smith.	Leg. Papilionoideae	2	1.4
"Erva doce"	*Pimpinella anisum* L.	Apiaceae	5	3.5
"Eucalipto"	*Eucalyptus globulus*	Myrtaceae	17	11.9
"Gengibre"	*Zingiber officinale* Roscoe	Zingiberaceae	2	1.4
"Gergilim"	*Sesamum orientale* L.	Pedaliaceae	1	0.7
"Girassol"	*Helianthus annuus* L.	Compositae	1	0.7
"Hortelã"	*Mentha* sp.	Labiatae	6	4.2
"Jatobá"	*Hymenaea courbaril* L.	Leg. Caesalpinoideae	1	0.7
"Laranja"	*Citrus aurantium* L.	Rutaceae	4	2.8
"Limão"	*Citrus limonia* Osbeck	Rutaceae	5	3.5
"Malva santa"	*Plectranthus barbatus* Andr.	Labiatae	1	0.7
"Malvarisco"	*Plectranthus amboinicus* Lour.	Labiatae	10	6.9
"Mastruz"	*Chenopodium ambrosioides* L.	Chenopodiaceae	6	4.2
"Melancia"	*Citrulus vulgaris* L.	Cucurbitaceae	1	0.7
"Mostarda"	*Brassica Intergrifolia* O. E. Schulz.	Cruciferae	1	0.7
"Pepaconha"	*Hybanthus ipecacuanha* (L.) Oken.	Violaceae	5	3.5
"Quebra-pedra"	*Phyllanthus* sp.	Euphorbiaceae	1	0.7
"Romã"	*Punica granatum* L.	Punicaceae	5	3.5
Total	------	------	143	100

* Information provided by the respondents.

From these 97 homemade preparations, 45 that were claimed by the population to resolve signs and symptoms that could be associated with an infectious condition were selected for tests (Table 3)

Table 2. List of the plants cited in the interviews, according to the part used, method of preparation, method of administration, and indication of use.

POPULAR NAME	PART USED	METHOD OF PREPARATION
“Abacaxi”	Fruit (pulp)	Syrup
“Agrião”	Flower	Tea (decoction)/L*
		Tea (decoction)*
“Alfavaca”	Leaf	Tea (decoction)
		Tea (decoction)/L*
		Tea (infusion)*
“Alho”	Bulb	Tea (decoction)/L*
		Tea (decoction)*
		Tea (decoction)
“Anador”	Leaf	Tea (infusion)
		Tea (decoction)
“Aroeira”	Bark	Tea (decoction)/L*
		Maceration
“Arruda”	Leaf	Tea (infusion)*
		Syrup
“Beterraba”	Tuber w/o peel	Tea (decoction)/L*
		Tea (decoction)
		Tea (decoction)
“Boldo”	Leaf	Tea (decoction)*
		Tea (infusion)
“Camomila”	Flower, above-ground part	Tea (decoction)
“Capim-santo”	Leaf	Tea (decoction)*
		Tea (decoction)
“Cebolinha branca”	Bulb	Tea (decoction)/L*
		Tea (decoction)
		Tea (decoction)
“Cidreira”	Leaf	Tea (decoction)*
		Tea (infusion)
		Tea (decoction)/G
		Juice*
“Corama”	Leaf	Tea (decoction)/L*
		Juice w/ milk*
		Tea (decoction)
“Cumaru”	Bark	Tea (decoction)/L*
“Erva doce”	Fruitlet	Tea (decoction)
		Tea (infusion)
		Tea (decoction)*
		Tea (decoction)/L*
“Eucalipto”	Leaf	Tea (decoction)*
		Tea (infusion)
		Tea (decoction)
		Tea (infusion)*
“Gengibre”	Rhizome	Mastication
“Gergilim”	Seed	Toasted/decoction*
“Girassol”	Seed	Toasted/decoction*
		Tea (infusion)
“Hortelã”	Leaf	Tea (infusion)*
		Tea (decoction)*
		Tea (decoction)/L*
“Jatobá”	Unripe fruit	Tea (decoction)/L
		Tea (decoction)
“Laranja”	Fruit (peel)	Tea (decoction)*
		Tea (infusion)
		Tea (infusion)*
“Limão”	Fruit (Juice and peel)	Tea (decoction)*
		Tea (decoction)
“Malva santa”	Leaf	Juice w/ milk*
		Tea (decoction)/L*
		Juice*
“Malvarisco”	Leaf, root	Syrup
		Juice w/ milk*
		Tea (decoction)
		Juice w/ milk*
		Juice w/ milk
“Mastruz”	Above-ground part (leaf)	Tea (decoction)/L*
		Tea (decoction)
		Tea (decoction)*
“Melancia”	Seed	Toasted/decoction*
“Mostarda”	Seed	Toasted/decoction*
“Pepaconha”	Root	Tea (decoction)/L*
		Maceration
“Quebra-pedra”	Root.	Tea (decoction)
“Romã”	Fruit (Seeds and peel)	Tea (decoction)/L
		Tea (decoction)

Syrup (*Lambedor*): Sugar + plant without adding water (whether heated on stove or not).
Tea (decoction)/L*: Syrup made by cooking the plant (mixture of plants).
Tea (decoction)/L: Syrup made by cooking the plant (plant used alone).
Tea (decoction)*: Plant is placed in boiling water (mixture of plants).
Tea (decoction): Plant is placed in boiling water alone.
Tea (infusion)/L*: Syrup made by steeping the plant (mixture of plants).
Tea (infusion)/L: Syrup made by steeping the plant (plant used alone).
Tea (infusion)*: Boiling water is added to the plant (mixture of plants).
Tea (infusion): Boiling water is added to the plant (plant used alone).
Juice*: Plant is homogenized in a blender with water and then strained (mixture of plants).
Juice w/ milk*: Plant is homogenized in a blender with milk and then strained (mixture of plants).
Juice w/ milk: Plant is homogenized in the blender with milk and then strained (plant used alone).
Maceration: Plant is mashed or chopped and steeped in cold water.
Mastication: Plant is chewed slowly.
Toasted/decoction*: Decoction made from roasted seeds and triturated plant (mixture of plants).

Table 3. Composition of samples tested for antimicrobial activity, method of preparation and indication of use, cited by the respondents.

Sample number	Sample Composition	Method of Preparation	Cited Indication of Use
1	Alfavaca + Eucalipto	Tea-D	Flu
2	Cidreira	Tea-D	Abdominal pain
3	Eucalipto + Alho + Limão	Tea-D	Flu
4	Mastruz + Malvariço + Leite	Juice	Abdominal pain
5	Eucalipto	Tea-D	Fever
6	Casca da laranja	Tea-D	Abdominal pain
7	Eucalipto + Alho	Tea-D	Sore throat
8	Eucalipto + Alfavaca + Limão	Tea-D	Flu/ cough
9	Gengibre	Mastication	Sore throat
10	Camomila	Tea-D	Abdominal pain
11	Erva doce	Tea-I	Abdominal pain
12	Casca de laranja + Boldo	Tea-D	Abdominal pain
13	Aroeira	Tea-D	Hygiene/infection
14	Pepaconha	Maceration	Cough/ shortness of breath
15	Cidreira	Tea-I	Fever
16	Quebra pedra	Tea-D	Kidney infection
17	Capim santo	Tea-D	Abdominal pain
18	Hortelã	Tea-I	Flu
19	Mastruz + Leite	Juice	Anti-inflammatory
20	Alfavaca	Tea-D	Expectorant
21	Corama + mastruz + malvariço	Juice	Heavy cough
22	Corama + malvariço	Syrup	Flu or cough
23	Malvariço + corama + mastruz + cebolinha branca + pepaconha	Syrup	Flu
24	Abacaxi	Syrup	Flu
25	Eucalipto + malvariço + cebolinha branca	Syrup	Cough
26	Beterraba	Syrup	Sore throat
27	Hortelã + malvariço	Syrup	Flu
28	Cumaru + eucalipto + hortelã + cebola + beterraba	Syrup	Sore throat
29	Romã	Syrup	Sore throat
30	Malva do reino	Syrup	Flu
31	Cebolinha + pepaconha + cumaru + beterraba + alho + mel de abelha	Syrup	Expectorant
32	Cebolinha branca + eucalipto + aroeira + pepaconha	Syrup	Cough
33	Pepaconha+ agrião + malvariço + alfavaca + cebolinha branca	Syrup	Expectorant
34	Corama	Tea-D	Flu/inflammation
35	Malvariço	Tea-D	Flu
36	Mastruz + leite	Tea-D	Flu
37	Romã	Tea-D	Cough
38	Cebola branca	Tea-D	Flu
39	Alho	Tea-D	Flu
40	Limão	Tea-D	Flu
41	Cidreira + goma	Tea-D	Abdominal pain
42	Jatobá	Syrup	Cough
43	Beterraba	Tea-D	Cough
44	Malvariço	Tea-D	Cough
45	Girassol + melancia + gergilim + mostarda + hortelã	Tea-D	Flu

Key: Tea-D = tea - decoction or boiling; Tea-I = tea - infusion

Antimicrobial activity

Table 4 shows the *in vitro* antimicrobial activity of the homemade preparations of the medicinal plants evaluated. Among the 45 samples tested, 55.6% (25 samples) showed inhibition of the growth of at least one of the microorganisms used. One of these samples (number 31) inhibited the growth of all the strains tested.

Table 4. Antimicrobial activity of the homemade preparations of medicinal plants determined by the well-diffusion method (inhibition zones in mm).

Sample no.	Microbial Strain					C +		C -
	SA[1]	EC[2]	PA[3]	SC[4]	CA[5]	A	C	H^2O
1	14	-	21	23	-	36	40	-
2	-	-	-	-	-	36	40	-
3	-	-	19	18	14	36	40	-
4	-	-	-	-	-	36	40	-
5	-	-	-	-	-	36	40	-
6	-	-	-	-	-	36	40	-
7	-	-	-	-	-	36	40	-
8	-	-	-	-	-	36	40	-
9	10	-	-	-	12	36	40	-
10	-	-	-	-	-	36	40	-
11	-	-	-	-	-	36	40	-
12	-	-	-	-	-	36	40	-
13	30	-	-	-	-	36	40	-
14	-	-	-	-	-	36	40	-
15	-	-	-	-	-	36	40	-
16	21	-	25	24	27	36	40	-
17	-	-	-	-	-	36	40	-
18	-	-	-	-	-	36	40	-
19	-	-	-	-	-	36	40	-
20	-	-	18	19	16	36	40	-
21	-	-	-	-	-	36	40	-
22	32	-	16	17	-	36	40	-
23	34	-	-	19	24	36	40	-
24	24	-	31	-	-	36	40	-
25	32	-	-	18	20	36	40	-
26	28	-	-	-	-	36	40	-
27	34	-	-	21	22	36	40	-
28	35	-	-	21	24	36	40	-
29	-	-	-	18	-	36	40	-
30	35	-	-	23	21	36	40	-
31	38	35	34	35	36	36	40	-
32	33	-	20	19	-	36	40	-
33	35	-	20	19	-	36	40	-
34	-	-	-	-	-	36	40	-
35	-	-	17	20	-	36	40	-
36	-	-	-	-	-	36	40	-
37	-	-	-	18	-	36	40	-
38	-	-	-	-	-	36	40	-
39	-	-	-	-	-	36	40	-
40	-	-	-	-	-	36	40	-
41	-	-	-	-	-	36	40	-
42	34	28	32	-	-	36	40	-
43	-	-	-	-	-	36	40	-
44	-	-	18	19	-	36	40	-
45	-	-	21	20	-	36	40	-

1: Staphylococcus aureus ATCC 6538P; 2: Escherichia coli ATCC 10536; 3: Pseudomonas aeruginosa ATCC 9027; 4:Salmonella enterica subsp. enterica serovar Choleraesuis ATCC 10708; 5 : Candida albicans ATCC 10231; C+ : positive control; C- : negative control; A: amikacin (30μg); C: ketoconazole (50μg). -: no inhibition zone.

DISCUSSION

This study shows that the use of plant preparations in folk medicine generally occurs with no or very little information of a scientific nature. Most consumers of herbal remedies believe that they are completely safe. People are frequently heard to say: "Even if it does no good, it won't do any harm," firmly believing that medicinal plants cannot damage one's health and that there are no contra-indications, since they are natural substances. Arnous et al., (2005) also found a strong belief in folk medicine, 83.6% of their interviewees stating that treatment with medicinal plants is effective.

The most commonly used part of the plant was the leaf, cited as the constituent in 60% of the preparations. The fruit of the plant ranked second, followed by roots, bark, and flowers, which were cited less frequently. A study conducted by Parente & Rosa (2001) presented somewhat different findings, in which there was a predominance of whole plant preparations. Regarding the methods used, herbal tea was mentioned in 74.2% of the preparations and, in 59.8%, the tea was made by decoction. Similar findings have been reported elsewhere (Arnous et al., 2005; Fuck et al., 2005; Parente & Rosa, 2001), which implies that in most cases, the plant is used erroneously, since only the hard parts (roots, stems and bark) should be cooked. According to Castellani (1999), infusion rather than decoction should be used with all soft parts of medicinal plants, such as leaves, flowers and buds, because they are rich in volatile components, delicate aromas and active ingredients that are degraded by the combined action of water and prolonged heating. These results thus indicate a need for the population to be informed on the correct ways of preparing the most commonly used medicinal plants.

In this study, the indications cited for the medicinal use of the plants generally included a wide range of health problems, the most outstanding of which related to the respiratory and digestive systems (71.1%). Similar results were obtained by Amorozo (2002) in a survey of ethnobotanical plants used therapeutically in the district of Santo Antonio do Leverger, MT, Brazil.

Antimicrobial activity

The study of the antimicrobial activity of plants held to be "medicinal", in the form of infusions and extracts, requires thorough investigation, since many factors hamper the verification of *in vitro* activity. Generally, natural products are complex and consist of undefined mixtures of active ingredients and secondary substances. Also, a variation in the plant composition can frequently be observed, which may enhance or antagonize the biological effect, or the composition may vary due to factors not associated with the plant itself, such as collection, storage and preparation. Furthermore, herbal products normally produce no effect in the short term and their cumulative effects generally take several weeks to be seen (Simões et al., 2003).

In reviewing the frequency with which some plant species are repeated in the preparations cited, we found that among the eight most widely used plants, five are known to have antimicrobial activity: *Plectranthus amboinicus*, *Eucalyptus globulus*, *Chenopodium ambrosioides*, *Ocimum gratissimum* and *Allium sativum* (Matos, 2002).

The main biological activity of *Eucalyptus globulus* occurs in the respiratory tract, on account of its essential oil that is rich in eucalyptol, which shows anti-flu, expectorant, and antiseptic activity in the respiratory tract, administered either orally or by inhalation (Sousa et al., 2004). However, in the Brazilian northeast, the oil of *Corymbia citriodora* is frequently used in folk medicine; this contains no eucalyptol, but rather citronellal. This substance causes

irritation in the mucous membranes of the respiratory tract and its antiseptic activity justifies its use as a cleansing agent (Matos, 2002).

E. globulus was cited in the preparation of eight homemade formulations, either alone or in association with other plants. When used alone, it showed no activity (sample 5 - Tables 3 and 4), possibly because it was prepared by boiling the leaves. Since its main active ingredient is a volatile substance, it may have been changed and lost its antimicrobial activity during prolonged exposure to heat. However, when coupled with *O. gratissimum* (sample 1 - Tables 3 and 4), even though prepared in an identical manner, the decoction showed better antimicrobial activity, probably due to the increased stability of eugenol, their main constituent (Sousa et al., 2004).

O. gratissimum (alfavaca or African basil) is widely encountered in tropical regions. It is used in folk medicine for upper respiratory tract infections, pneumonia, cough, fever and conjunctivitis. The essential oil extracted from this plant has several active compounds, the most outstanding of which are cineole (eucalyptol) and eugenol. Some studies have found antimicrobial activity in *O. gratissimum* against *Staphylococcus aureus*, *Shigella flexneri*, *Salmonella enteritidis*, *Escherichia coli*, *Klebsiella sp*., *Proteus mirabilis* and *Pseudomonas aeruginosa* (Nakamura et al., 1999; Pereira et al., 2004).

In this study, there was a change in the antimicrobial activity of the homemade preparations containing alfavaca. Like the eucalypt, this plant also contains volatile active substances that can be lost through heating, consequently reducing its antimicrobial activity. Moreover, the contents of these active substances (eugenol and eucalyptol) vary in the plant during the day (Matos, 2002), suggesting that its inhibitory activity on microorganisms may also be altered by the time of day the plant was collected. Gobbo-Neto and Lopes (2007) observed that eugenol concentration in the *O. gratissimum* essential oil varies by more than 80% during the day, the best time to collect the plant being 12 noon (98% of the essential oil) and the worst at 5 p.m. (11% of the essential oil).

Highlighted among the therapeutic properties of *A. sativum* (garlic) are the following: it is hypolipidemic, anti-clotting, antitumor, and anti-infectious (Alonso, 1998). The compound ajoene is responsible for the protection against thrombosis and the reduced levels of cholesterol and fats in the blood. Its anti-infectious action is due to the presence of a related substance, allicin, which destroys groups essential to the proliferation of bacteria. However, the maceration and boiling of the garlic quickly break down its active ingredients. Thus, it is important to practice the correct use of garlic, which should be restricted to unheated, recently obtained preparations, preferably preserved at an acid pH (Sousa et al., 2004).

The results showed that garlic, when heated — i.e., in the form of a decoction (samples 7 and 39 – Tables 3 and 4) — showed no antimicrobial activity against any of the microbial strains tested. However, when *Citrus limonia* (lemon) was added to *E. globulus* tea with *A. sativum* (sample 3 – Tables 3 and 4), even in the presence of heat, the outcome was improved, since three out of the five microorganisms tested were inhibited. In this case, it is likely that the antimicrobial potential of *A. sativum* was stabilized by the acid pH introduced by the presence of the lemon juice in the mixture.

In general, all the samples used in the form of syrup (*lambedor*) had good antimicrobial activity, not only those prepared by boiling the plant material and adding sugar or honey to the decoction (samples 22, 23, 25, 27, 28, 29, 31, 32, 33 and 42), but also those in which sugar was mixed directly with the plant, without adding water (samples 24, 26 and 30). According to some respondents, medicinal plants are used together in the same preparation, such as syrup, in order to increase the effectiveness of the treatment. However, it must be pointed out that the promising results obtained *in vitro* may be due to the high concentration of sugar or honey in the formulation.

In the studied population, the use of medicinal plants as a low-cost alternative for the treatment of disease was quite frequent, not only because the users rely on the healing power of these plants, but also because they believe that such plants cannot be harmful to the health, because they are natural. However, it was found during the study that several factors may compromise the effectiveness of the treatment, or even its safety. These factors range from the misidentification of plant species to the incorrect use of formulations. The inconsistent indications of herbal remedies, whose preparation and purpose were sometimes at odds with the scientific literature, are also an important factor. Therefore, there is a need for further discussions with such communities to clarify these questions, so that risks can be avoided.

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RESUMO

Determinação in vitro do potencial antimicrobiano de preparações caseiras de plantas medicinais utilizadas para o tratamento de doenças infecciosas

Grande parte da população de países em desenvolvimento utiliza plantas ou preparações vegetais nos cuidados básicos à saúde. Muitas das espécies vegetais utilizadas na medicina popular apresentam propriedades antimicrobianas comprovadas; no entanto, diversos fatores, como a metodologia incorreta de preparo, podem interferir na eficácia do tratamento. O presente trabalho teve como objetivo avaliar a utilização de preparações caseiras de plantas medicinais para o tratamento de doenças infecciosas através da determinação *in vitro* do potencial antimicrobiano. A partir de informações contidas em questionários que foram previamente aplicados à população participante, as amostras foram preparadas de forma semelhante e analisadas através do método de difusão em ágar. Foram entrevistadas 41 famílias, das quais 97,6% afirmaram utilizar as plantas medicinais como opção terapêutica no tratamento de doenças. Em um total de 39 questionários, foram citadas

97 diferentes preparações caseiras de plantas. Das 45 amostras (indicadas para tratar algum sinal ou sintoma que podem estar relacionado a um quadro infeccioso) submetidas à avaliação da atividade antimicrobiana *in vitro,* 55,6% (25 amostras) apresentaram atividade inibitória sobre o crescimento de pelo menos um dos microrganismos utilizados. A maioria das plantas com propriedade antimicrobiana conhecida e que foram citadas pelos entrevistados apresentaram variações na sua atividade *in vitro* de acordo com o modo como foram preparadas.
*Palavras-chave***:** Plantas Medicinais. Produtos naturais com ação antimicrobiana. Medicina tradicional.

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